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97. Quantitative Analysis of Triacylglycerols and Other Lipid Classes in Edible Fats and Oils *via* HPLC-ELSD*

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Edible fats and oils are typically comprised of >99% triacylglycerols. However, certain plant oils such as those extracted from rice bran and corn fiber can have much higher levels (5–10 % and 20–40%, respectively) of non-triacylglycerol lipids. Methods are needed to simultaneously analyze the levels of triacylglycerols and other lipids in edible fats and oils. Using a gradient of hexane-isopropanol-acetic acid, a bonded-phase DIOL column, and an evaporative light-scattering detector, we have developed a rapid HPLC method to quantitatively analyze the nonpolar lipid classes of bran and fiber oils. We have used this method to analyze the lipids in corn fiber oil and rice bran oil. The levels of ferulate esters were four times higher in corn fiber oil than in rice bran oil. The ferulate esters (oryzanols) in rice bran oil have been shown to lower serum cholesterol in laboratory animals and humans. This new HPLC method should be useful for the quantitative analysis of nonpolar lipids in many edible fats and oils.

97.1 Introduction

Our laboratory (1,2) and others (3) have published numerous HPLC methods for the analysis of the total lipid extracts from plant, animal, and microbial cells and tissues. In this study we investigate the use of these methods for the quantitative analysis of lipid classes in edible fats and oils, with special emphasis on rice bran oil and corn fiber oil. Because existing HPLC methods for lipid class analysis were not satisfactory for the separation of nonpolar lipids in these samples, a new method was developed to attempt to improve these separations. Most previous methods for the HPLC analysis of nonpolar lipids in edible fats and oils have utilized UV or fluorescence detectors and have only analyzed those lipids that have strong chromophores or fluorophores (4,5). The current method employs an evaporative light-scattering detector (ELSD), which is able to accurately detect all lipids.

97.2 Materials and methods

Rice bran oils (crude, alkali refined, and physically refined) were a generous gift of Dr. Eugene Rogers, University of Massachusetts, Lowell, MA, USA. Corn fiber oil (crude) was prepared by shaking 4 g common corn fiber (from yellow dent #2 corn) ground to 20 mesh with a Wiley Mill (Thomas Scientific, Philadelphia, PA), with 40 ml hexane, in a 55 ml screw-top tube for 1 h. The lipid extract was then filtered through a 5.5 cm Whatman GF/A, glass microfiber filter and injected directly into the HPLC. A separate sample (8 g) of corn fiber was extracted with supercritical CO_2 (25 min at 2.5 L/min,10,000 psi, and 40°C) in an Applied Separations (Allentown, PA, USA) *Spe-ed* Supercritical Fluid Extractor. The supercritical fluid-extracted oil was dissolved in hexane and injected into the HPLC. Butylated hydroxytoluene (0.01%) was added to all samples to prevent oxidation.

The HPLC system used for the separation of nonpolar and polar lipid classes consisted of an ISCO (Lincoln, NE) Model 2350 pump, an ISCO Model 2360 gradient programmer, an Alcott (Norcross, GA) Model 738 autosampler, an ISCO Model V_4 UV detector operated at 205 nm, and an Alltech-Varex (Deerfield, IL) Mark III Evaporative Light Scattering Detector (ELSD). The column was a LiChrosorb 5 Si 60 (3 x 100 mm) from Chrompack, Inc. (Raritan, NJ), at a flow rate of 0.5 ml/min. The solvents were: A, hexane; B, isopropanol; and C, 0.04% triethylamine in water, (C was prepared fresh daily). The linear gradient timetable was: At 0 min, 100/0/0; at 5 min, 95/5/0; at 10 min, 85/15/0; at 15 min, 40/60/0; at 53 min, 40/51/9; at 68 min, 40/51/9; at 73 min, 40/60/0; at 78 min, 100/0/0; at 100 min, 100/0/0; (%A/%B/%C, respectively).

The HPLC system used for the separation of nonpolar lipid classes consisted of a Hewlett Packard (Avondale, PA, USA) Model 1050 ternary gradient system (HPLC pump, autosampler, and UV/visible detector), and an Alltech-Varex Mark III ELSD. The column was a LiChrosorb DIOL, 5 µm, (3 × 100 mm), from Chrompack, Inc., and the flow rate was 0.5 ml/min. The solvents were: A, hexane/acetic acid, 1000/1, v/v; and B, hexane/isopropanol, 100/1, v/v, (Both were mixed fresh daily to eliminate variability caused by evaporation and/or absorption of moisture). The linear gradient timetable was: At 0 min, 100/0; at 8 min, 100/0; at 10 min, 75/25; at 40 min, 75/25; at 41 min, 100/0; at 60 min, 100/0; (%A/%B, respectively).

97.3 Results and discussion

Our previously-published normal-phase HPLC system for plant lipid class analysis provided adequate separation and quantification of the low levels of glycolipids and phospholipids in the total lipid extract from rice bran oil (Figure 97.1) and corn fiber oil (Figure 97.2). In these samples only trace levels of glycolipids were detected, and the PC (see legend to Table 97.1 for a list of abbreviations) in rice bran oil was the only phospholipid that was quantitatively detected (Table 97.1). When corn fiber oil was prepared by hexane extraction, there were small but detectable peaks of SG, PE, and PC. However, when corn fiber oil was prepared by supercritical fluid extraction, there were no detectable peaks of any glycolipids or phospholipids (data not shown). Since a majority of the lipids in these samples are nonpolar, and these types of lipids are not adequately separated in this system, we chose to explore a new HPLC method to separate nonpolar plant lipids.

When developing a new system for the separation of plant nonpolar lipids, we chose to use a DIOL bonded-phase column because of its durability. We also achieved better separation of nonpolar lipids if we dissolved the samples in very nonpolar solvents such as 100% hexane (and 0.01% BHT). These two new procedures are contrary to those used in our previous ternary gradient lipid class HPLC system (Figures 97.1–97.2), where we have found that: a) injecting the sample in more polar solvent (such as 85:15 chloroform/methanol) is necessary in order to

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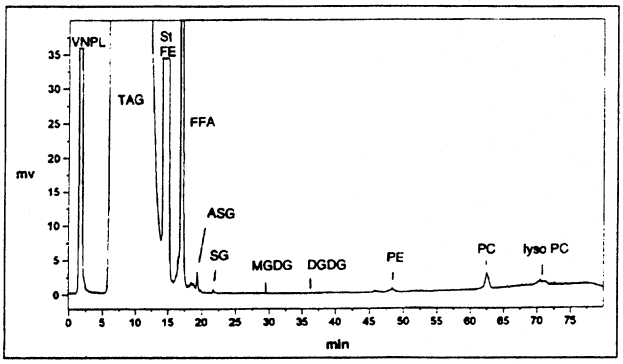

Fig. 97.1 Chromatogram showing the separation of nonpolar and polar lipid classes in rice bran oil (210 µg crude lipid was injected) with our ternary lipid class HPLC-ELSD system

The abbreviations are defined in Table 97.1.

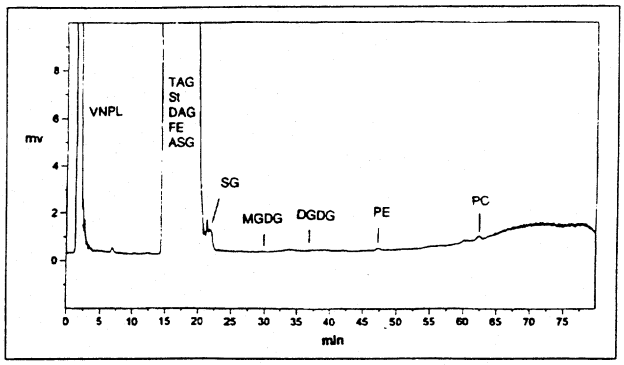

Fig. 97.2 Chromatogram showing the separation of nonpolar and polar lipid classes in corn fiber oil (hexane-extracted, 170 µg crude lipid was injected) with our ternary lipid class HPLC-ELSD system

The abbreviations are defined in Table 97.1.

solubilize some glycolipids and phospholipids, and b) silica gel columns seem to provide better separation of the glycolipids and phospholipids than DIOL or other bonded-phase columns.

The new HPLC method for the separation of plant nonpolar lipids is a very gradual binary gradient that starts with an eight minute step of hexane-acetic acid (100/0.1) and proceeds to a thirty minute step of hexane-isopropanol-acetic acid (99.65:0.25:0.10). This method achieved baseline separation of nine identifiable components in rice bran oil (Figure 97.3) and corn fiber oil (Figure 97.4). The chromatograms of the nonpolar lipids in corn fiber oil prepared by hexane extraction (Figure 97.4) *vs* supercritical fluid extraction (data not shown) were identical. In these chromatograms there was some separation of the individual fatty acids (the order of elution was stearic, palmitic, oleic, and linoleic acids) and of the molecular species of 1,3- and 1,2-diacylglycerols. Sterols were similarly resolved into two separate baseline-resolved peaks. However, for quantification, the total fatty acids, total sterols, and total 1,3- and 1,2-diacylglycerols were reported. Standard curves of mass (1–25 µg) vs peak area, for each of the nine identifiable lipid classes, were constructed and used for quantitative analysis of nonpolar lipid classes in the samples (Table 97.1). γ– tocopherol was quantified using the UV detector and all other

TABLE 97.1 A comparison of the major nonpolar and polar lipids in corn fiber oil and rice bran oil; the PC was quantitatively analyzed using the gradient system in Figures 97.1 and 97.2; the other lipid classes were quantitatively analyzed using the gradient system in Figures 97.3 and 97.4

Sample	StE	TAG	FFA	γ-toc	St	1,3-D	FE	1,2-D	PC
	µg/100 µg oil								
Rice bran oil (crude)	2.37	88.66	2.74	0.050	0.95	2.45	1.50	1.00	0.28
Rice bran oil (phys ref.)	2.31	90.34	0	0.043	2.24	2.42	1.44	1.21	0
Rice bran oil (alk ref.)	2.19	94.67	0	0.037	1.30	1.22	0	0.58	0
Corn fiber oil (crude)	7.91	72.88	3.54	0.580	3.70	3.05	5.66	2.68	tr

Abbreviations: phys ref., physically refined; alk ref., alkali refined; VNPL, very nonpolar lipids; StE, sterol-fatty acyl esters; TAG, triacylglycerols; α-toc, α-tocopherol; FFA, free fatty acids; γ-toc, γ-tocopherol; St, free sterols; DAG, diacylglycerols; 1,3-D, 1,3-diacylglycerols; FE, ferulate esters; 1,2-D, 1,2-diacylglycerols; ASG, acylated sterol glycoside; SG, sterol glycoside; MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; PE, phosphatidylethanolamine; PC, phosphatidylcholine; lyso-PC, lyso-phosphatidylcholine

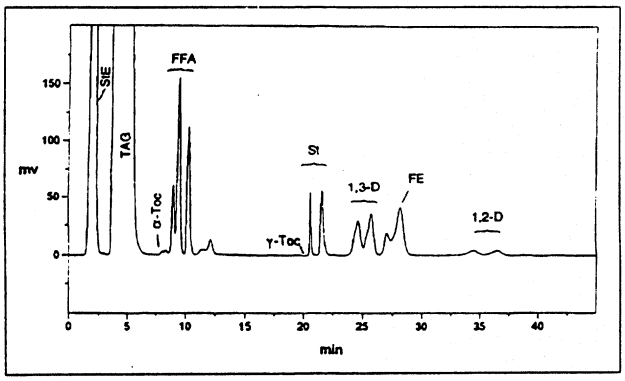

Fig. 47.3 Chromatogram showing the separation of nonpolar lipid classes in rice bran oil (210 µg crude lipid was injected) with our new nonpolar lipid binary HPLC-ELSD system.

The abbreviations are defined in Table 97.1.

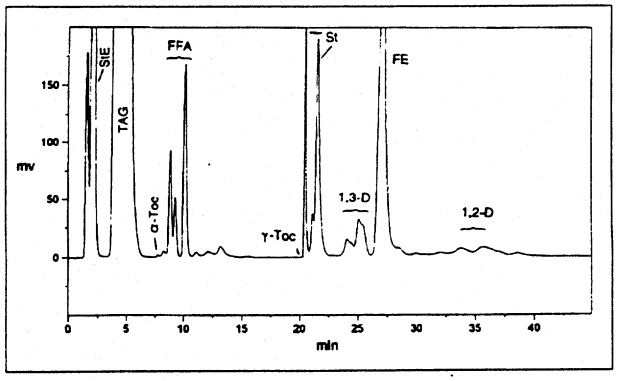

Fig. 97.4 Chromatogram showing the separation of nonpolar lipid classes in corn fiber oil (hexane-extracted, 170 µg crude lipid was injected) with our new nonpolar lipid binary HPLC-ELSD system

The abbreviations are defined in Table 97.1.

components were quantified using the ELSD. Two other samples of refined ricebran oil (alkali refined and physically refined) were also quantitatively analyzed using this new nonpolar lipid system (chromatograms not shown) and the quantitative results are summarized in Table 97.1. Previous studies have shown that alkali refining of rice bran oil successfully removed the free fatty acids, but it also destroyed the ferulate esters (oryzanols), which have been shown to impart rice bran oil with hypocholesterolemic properties. In order to preserve the ferulate esters and still remove the free fatty acids, Nicolosi *et al.* (6) have developed a process called "physical refining" which accomplishes this goal (Table 97.1).

Corn fiber oil (crude) contains most of the same lipid classes as rice bran oil (crude) and it appears to contain about fourfold higher levels of ferulate esters (oryzanols). Oryzanols have previously been shown to be active hypocholesterolemic agents in rice bran oil (6). Our laboratory is in the process of performing collaborative animal feeding studies to determine the possible hypocholesterolemic properties of corn fiber oil. Although most other edible fats and oils do not contain as many non-triacylglycerol components as rice bran oil and corn fiber oil, this new HPLC methodology should also prove valuable for their quantitative analysis.

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